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INSTAR NUMBER AND LARVAL DEVELOPMENT IN *LYCAENA PHLAEAS HYPOPHLAEAS* (BOISDUVAL) (LYCAENIDAE)

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ABSTRACT. The arctic-alpine butterfly *Lycaena phlaeas hypophlaeas* (Boisduval) may have either four or five larval instars, the number apparently being fixed at oviposition. Factors affecting instar number were investigated in a laboratory colony of *L. p. hypophlaeas* from the White Mountains of California. Adults in oviposition cages were subjected to outdoor ambient conditions of day-length and temperature, but larvae were reared indoors under nearly constant conditions (ca. 16 h light, 25°C). Larvae with five instars predominated when oviposition occurred during short days (<11 h light) and low maximum diurnal temperatures (ca. 22°C). When oviposition occurred during longer days (>12 h light) and higher mean diurnal temperatures (ca. 33°C) most larvae had four instars. Larvae having five instars required about 70% longer to mature than larvae having four instars. Although diapause is not obligate, overwintering probably occurs as larvae, which are more resistant to cold than are pupae and adults.

Additional key words: diapause, Lycaeninae, *Oxyria digyna*.

The primarily holarctic lycaenid butterfly *Lycaena phlaeas* (L.) inhabits a wide range of habitats from sea level to ca. 4000 m elevation. Various subspecies of *L. phlaeas* in Asia, Europe, and eastern North America are multivoltine, polyphagous (primarily on *Rumex* species), and common at low elevations. However, *L. p. hypophlaeas* (Boisduval) of western North America is univoltine, apparently monophagous on *Oxyria digyna* (L.) Hill, and confined to arctic-alpine habitats (Ferris 1974). This subspecies occurs in isolated colonies above 3000 m in the central Sierra Nevada (Bishop Pass to Sonora Pass) and White Mountains of California; collection records indicate a flight period from mid-July to early September (Shields & Montgomery 1966, Ferris 1974). Its habitat is one of extreme (especially cold) and unpredictable weather for much of the year; even in summer there may be frost and occasional snow. The restricted range of this subspecies is puzzling since suitable hosts (*Rumex* spp.) are widespread at lower elevations in California where they are utilized by other *Lycaena* species (Ballmer & Pratt, 1988). Also puzzling is the fact that, unlike other California Lycaeninae, *L. p. hypophlaeas* may have either four or five instars (Ballmer & Pratt, 1988). Investigations reported here concerning the biology of *L. p. hypophlaeas* were undertaken primarily to clarify instar number under controlled environmental conditions. Additional observations on growth rate and cold tolerance of stages may help explain the ability of this butterfly to survive in the arctic-alpine zone.

MATERIALS AND METHODS

A laboratory culture of *L. p. hypophlaeas* was derived from progeny of a single female captured in the White Mountains (California, Mono Co., White Mt., elev. ca. 4000 m, 26 July 1987) by J. F. Emmel. A single mature larva was also found on *Oxyria digyna* (same data) by G. F. Pratt. No other likely host was encountered at the collection site, although at a lower elevation (3300 m) a few km away, *Rumex paucifolius* Nutt. ex Wats. was abundant and utilized as a larval host by *Lycaena cupreus* (W. H. Edwards) and *L. editha* (Mead). One of us (G.F.P.) has also found larvae of *L. p. hypophlaeas* on *Oxyria digyna* in the nearby Sierra Nevada (Mono Co., Mt. Dana, elev. 3600 m, 7 August 1985). *Oxyria digyna* is also a host for other arctic-alpine populations of *L. phlaeas* in North America (Ferris 1974, Harry 1981).

In captivity, adult butterflies were confined with *Rumex crispus* L. and *R. acetosella* L. in a screened cage (0.3 m × 0.3 m × 0.3 m) for mating and oviposition. The cage was outdoors in a sheltered location with partial sun exposure. Ova were brought indoors and larvae were reared in quart (0.95-l) plastic food containers on leaves of both *R. acetosella* and *R. crispus*. Pupae were transferred to the screened cage for eclosion.

Nineteen neonatal larvae from ova produced during the first week of September (long-day ova) were placed individually in 7-dram (25-ml) plastic vials and reared on leaves of *R. crispus*. Leaves were replaced as needed (usually every 2–3 days for young larvae and daily for last instars). A second group of 23 neonates from ova produced at the end of October (short-day ova) was reared under slightly different conditions. These larvae were kept individually in 25-dram (90-ml) plastic vials with two 25-mm-diam. screened ventilation openings, and fed leaves of *R. crispus*. A small hole in each lid allowed the leaf stem to protrude for immersion in water contained in a second vial; this permitted leaves to remain fresh longer while the ventilation prevented mold which occasionally appeared in the smaller nonventilated vials used earlier. All larvae were reared indoors at 25 ± 3°C (brief temperature fluctuations resulted from operation of indoor heating and cooling equipment). Larvae were inspected daily for signs of ecdysis. Dates of ecdysis were recorded for each larva, and head capsules were measured using a microscope and ocular micrometer.

Total illumination from indirect natural daylight and artificial lighting from overhead fluorescent lamps exceeded 16 h per day. Only adults and ova were exposed to natural (outdoor) diurnal photoperiods and temperatures. There were 12.5 h of daylight (sunrise to sunset) on 10 September, the mean eclosion date for long-day ova, and 10.75 h

of daylight on 3 November, mean eclosion date for short-day ova; the effective period of daylight on both dates may have been somewhat longer. Mean maximum and minimum diurnal temperatures for the seven days preceding mean eclosion dates were 33.3°C and 13.3°C, respectively, for long-day ova and 21.5°C and 12.8°C, respectively, for short-day ova.

Four mature larvae from long-day ova were preserved and the remainder allowed to pupate. Four pupae were refrigerated at 5°C for 28 days to test the effect of mild but prolonged chilling. All larvae from short-day ova were reared to adults without chilling.

Other experiments tested the effect of extreme chilling on additional larvae, pupae, and adults. Ten second instars and 12 fourth instars (all destined to have 5 instars) were removed from the colony during December (from short-day ova) and placed in 25-dram (90-ml) ventilated vials, as described above, with fresh host leaves.

Vials were refrigerated (5°C) for 21 days, then wrapped in damp paper towels and placed inside larger sealed jars which were kept at −7°C for 28 days. After the freezing treatment, jars were allowed to thaw at 5°C for 24 h; the vials were then removed, larval condition was assessed, and survivors were given fresh host leaves; rearing continued at 25°C. Ten pupae were similarly treated (7 days at 5°C followed by 28 days at −7°C).

While refrigerated at 5°C, second instars fed considerably, but fourth instars did not feed. Leaves damaged by feeding were dried in a press, weighed, and photocopied. The paper images were cut out and weighed; then their feeding-damaged portions were cut out and weighed to determine percent of feeding damage. The latter values were then used to calculate quantity of leaf tissue eaten per larva.

On several occasions it was noted that brief periods (1–6 h) of exposure to −7°C were not lethal to adults; but death usually occurred after 2–3 consecutive exposures of such duration. The effect of milder but more prolonged exposure to cold was tested by refrigerating 13 freshly eclosed adults at 5°C for 30 days. Adults were placed individually in 25-dram (90-ml) ventilated vials which were then placed inside plastic bags with damp paper towels and refrigerated.

Statistical significance of differences in head size and instar duration was determined by *t*-tests.

RESULTS

Instar number and duration. Mean duration of each larval instar and overall larval and pupal stages are presented in Table 1. Only one male and six females from long-day ova are included owing to loss of

gender data for the remainder; therefore, discussion of sex-correlated differences in development rates is restricted to larvae from short-day ova. In general, females developed more rapidly than males, especially in the third and 'extra' instars (fourth instar of five-instar larvae). Sex-correlated differences in development times among larvae from short-day ova were most significant for third instars (P = 0.02 and 0.051 for four- and five-instars, respectively); for other larval instars P ranged from 0.13 to 0.84. Mean larval stage duration of five-instar larvae was greater than that of four-instar larvae; the difference for males (ca. 35% greater) is not significant (P = 0.08), but for females (ca. 49% greater) it is (P = 0.008).

Most larvae from long-day ova (17 of 19) had four instars, and required a mean of 23 days to pupate (both sexes combined); one larva had five instars, and another, which died of a whitish fungal infection in the fourth instar, would have molted again judging from its head size had it survived. Short-day ova produced mostly five-instar larvae (17 of 23), and required a rounded mean of 27 days to pupate (both sexes combined); remaining larvae had five instars and required a rounded mean of 41 days to pupate. Nevertheless, there was considerable individual variation and some overlap in developmental time ranges. It is remarkable that among both four- and five-instar larvae, some individuals remained as larvae about twice as long as others; range of larval duration for all larvae was 13–59 days. No significant differences in pupal duration were found with respect to sex, number of larval instars, or day length.

Head size. Measurements of head widths indicate no significant sex-related differences (P = 0.47, 0.21, 0.40, and 0.55 for instars 1, 2, 3, and 4, respectively, of five-instar larvae from short-day ova). There were also no significant differences in mean head size between four- and five-instar larvae from short-day ova for instars 1, 2, and 3 (P = 0.39, 0.54, and 0.78, respectively). Therefore, data were pooled for all larvae in comparing head sizes of first, second, third, and 'extra' instars of larvae conceived under long- and short-day conditions (Table 2). Since most larvae were reared to pupation, and the last-instar head capsule was invariably deformed at pupation, the head widths of last-instar larvae included in Table 2 are based primarily on preserved larvae reared concurrently. The mean first-instar head width of larvae from short-day ova was slightly but significantly (P = 0.015) larger than that of long-day ova. The head size of the 'extra' (fourth) instar of five-instar larvae was intermediate between that of third and last instars; thus, some growth occurred in all instars.

Values presented here for head size should not be considered typical of all populations of *L. p. hypophlaeas*. The mean last-instar head width

TABLE 1. Larval and pupal duration of *Lycaena phlaeas hypophlaeas* indoors at 25°C.

Sex	No. larval instars	N	Mean no. days ± SD of larval instars					Mean larval stage (days ± SD)[1]	Mean pupal stage (days ± SD)
			First	Second	Third	'Extra'	Last		
Long-day ova									
Male	4	1	4.0	6.0	8.0	—	5.0	23.0	8.0
Female	4	6	3.3 ± 0.5	6.0 ± 1.7	5.2 ± 1.5	—	6.0 ± 1.3	20.5 ± 4.0	8.2 ± 1.2
Short-day ova									
Male	4	2	4.5 ± 0.7	7.0 ± 0	13.5 ± 0.7	—	7.5 ± 0.7	32.5 ± 0.7	8.0 ± 0
Female	4	4	5.0 ± 2.2	6.8 ± 1.9	6.0 ± 2.6	—	6.3 ± 1.0	24.0 ± 6.1	7.8 ± 0.5
Male	5	12	4.5 ± 0.7	7.8 ± 1.9	13.2 ± 1.8	11.1 ± 6.7	7.3 ± 1.8	43.8 ± 8.2	8.8 ± 1.0
Female	5	5	4.2 ± 0.5	8.0 ± 1.2	11.2 ± 1.6	6.2 ± 3.2	6.2 ± 0.8	35.8 ± 3.4	8.2 ± 0.8

[1] Egg hatch to pupation.

TABLE 2. Mean head width of *Lycaena phaeas hypophlaeas* larvae reared indoors at 25°C.

Oviposition day length	Mean head width ± SD of larval instars									
	First	N	Second	N	Third	N	'Extra'	N	Last	N
Long	0.24 ± 0.01	14	0.43 ± 0.02	17	0.72 ± 0.06	18	0.89 ± 0.01	2	1.25 ± 0.04	8
Short	0.26 ± 0.01	21	0.43 ± 0.02	23	0.72 ± 0.03	23	0.87 ± 0.03	17	1.22 ± 0.04	5

(1.46 mm) of 10 *L. p. hypophlaeas* larvae from Mount Dana in the Sierra Nevada is 17% larger than that of 13 larvae from the White Mountain colony (1.24 mm); this difference is highly significant (P = 0.00002).

Cold exposure. The mortality rate from freezing was similar for second- and fourth- ('extra') instar larvae. Six (of 10) second instars exposed to −7°C for 30 days survived, as did 7 (of 12) fourth instars exposed to the same conditions. During 21 days of exposure to 5°C (before freezing), second instars consumed a mean 0.47 cm^2 (0.002 mg, dry wt.) of leaf tissue.

Adults and pupae were less tolerant of cold. Although all 4 pupae exposed to 5°C for 28 days survived and produced adults, only 1 of 10 pupae frozen (−7°C) for 28 days eclosed, and it was unable to properly expand its wings. All 13 adults refrigerated at 5°C for 30 days perished.

CONCLUSIONS

Instar number in *Lycaena p. hypophlaeas* is variable; four instars are prevalent under warm, long-day (late summer) conditions while five instars predominate when days are cooler and shorter in fall. Number of instars is apparently fixed at oviposition. Developmental time is greater for five-instar larvae than for four-instar larvae. Similar lengthened larval development and extra molts, but without apparent growth, in response to short day-length have also been reported in the multivoltine *L. p. daimio* Seitz of Japan (Sakai & Masaki 1965, Endo et al. 1985).

The greater development time required for *L. p. hypophlaeas* larvae produced under short-day conditions is probably important in winter survival. Unlike at least most other California *Lycaena* species, *L. p. hypophlaeas* does not have an obligate diapause. However, an extended larval duration induced by short day-length and further promoted by reduced activity due to cold fall and winter temperatures reduces the possibility of premature maturation and subsequent exposure of the less cold-tolerant stages to winter conditions. The great variability in development time probably also contributes to survival, since it ensures that some individuals are likely to be in the most cold-tolerant (larval) stage at all times of the year.

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